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Review Article

Different methods of extraction of bioactive compounds and their effect on biological activity: A review

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Abstract: As of yet, there isn't a single technique that is accepted as the standard for extracting bioactive chemicals from plants.

Methods. The effectiveness of both traditional and unconventional extraction methods largely depends on key input variables, knowledge of the composition of plant matter, bioactive chemical chemistry, and scientific knowledge.

Results. The necessity for the most suitable and standardized technology to separate active ingredients for plant matter is highlighted by the utilization of bioactive chemicals in several economic sectors, including the pharmaceutical, food, and chemical industries. This review aimed to discuss there are several extraction methods and their basic mechanisms for the extraction of bioactive substances from medicinal plants.

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1. INTRODUCTION

The choice of the best extraction technique is crucial to both quantitative and qualitative studies of bioactive chemicals derived from plant sources (Smith, 2003; Sasidharan *et al*., 2011). The initial step in every study of a medicinal plant is extraction, which has a substantial impact on the outcome. "Sample preparation techniques" is another name for extraction methods. The majority of the time, this area of research is ignored and carried out by untrained research staff (Azmir *et al*., 2013), despite the fact that sample preparation techniques take up about two-

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thirds of an analytical chemist's time and effort. According to a study (Huie, 2002), the majority of academics think sample preparation is crucial for any analytical research.

The success of bioactive compound analysis is still largely dependent on the methods of extraction, the factors used as input, and the specifics of the plant sections (Moldoveanu & David, 2002). However, it is true that contemporary spectrometric and chromatographic methods have made the process easier than before. Matrix characteristics of the plant component, pressure, temperature, solvent, and time are the most frequent variables influencing extraction operations (Hernández *et al*., 2009). The development of bioactive analysis over the past ten years has been fueled in large part by our growing understanding of the various bioactive compounds' dynamic chemical nature (Dar *et al*., 2015). The pharmaceutical, food additive, and even natural pesticide industries have developed an interest in bioactive compounds derived from natural sources as a result of these enormous technical and technological advancements (Uwineza & Waśkiewicz, 2020). Bioactive substances typically coexist with other substances found in plants. Different plant components, including leaves, stems, flowers, and fruits, can be used to identify and characterize bioactive chemicals. Various extraction techniques can be used to extract plant components. Over the past fifty years, novel techniques that are more ecologically friendly have been developed because they utilize fewer synthetic and organic chemicals, operate more quickly, and provide extracts of higher yield and quality. Ultrasound (Azmir *et al*., 2013), pulsed electric field (Toepfl *et al*., 2006), enzyme digestion (Gaur *et al*., 2007), extrusion (Dunford, 2008), microwave heating (Kaufmann & Christen, 2002), ohmic heating (Lakkakula *et al*., 2004), supercritical fluids (Ghafoor *et al*., 2010), and accelerated solvents (Smith, 2003) have all been investigated as novel strategies to improve the production and selectivity of bioactive components in plant products. Traditional extraction methods like Soxhlet are still used as a benchmark when evaluating the efficacy of newly developed methodologies. There are numerous scientific publications, book chapters, and monographs where non-traditional methods have been thoroughly examined (Wang & Weller, 2006). These works emphasize the application of extraction methods for food additives, nutraceuticals, and many other industries, but they do not discuss the extraction of bioactive chemicals by herbal plants. The goal of the current review is to give a thorough analysis of several methods for extracting bioactive substances from medicinal plants.

2. BIOACTIVE COMPOUNDS

Since the dawn of humankind, plants have been employed by humans. Initially, people just used plants for food, but once their medical capabilities were discovered, this natural flora started to be employed by many different human cultures as a source for disease treatment and health improvement. Through thousands of recipes, Egyptian papyrus demonstrated the value of coriander and castor oil as preservatives, cosmetics, and medicines (Vinatoru, 2001). Hippocrates, Theophrastus, Celsus, Dioscorides, and many other scholars from the Roman and Greek eras reported tens of thousands of medicinal applications for plants (Paulsen, 2010). For a very long time, Romanians have been known for using medicinal herbs. For instance, in his writings from the fifth century B.C., Herodotus wrote that the people who lived north of the Danube River employed the herb Leonurus cardiaca (Motherwort). The Romanian pharmacopeia introduced herbal products in the 19th century, and Cluj city became home to the first institute specialized in medicinal herbs in 1904 (Vinatoru, 2001). The history of bioactive compounds is clearly illustrated by the ancient use of herbal plants. People did not know about bioactive molecules in the past, although there were several applications for these substances in various fields. Typically, secondary metabolites are how plants make their bioactive substances (Bernhoft, 2010). Every living thing, from single-celled bacteria to about a million cellular plants, processes a different set of chemical compounds in order to survive and live. All biological compounds can be separated into two main classes. The first are primary

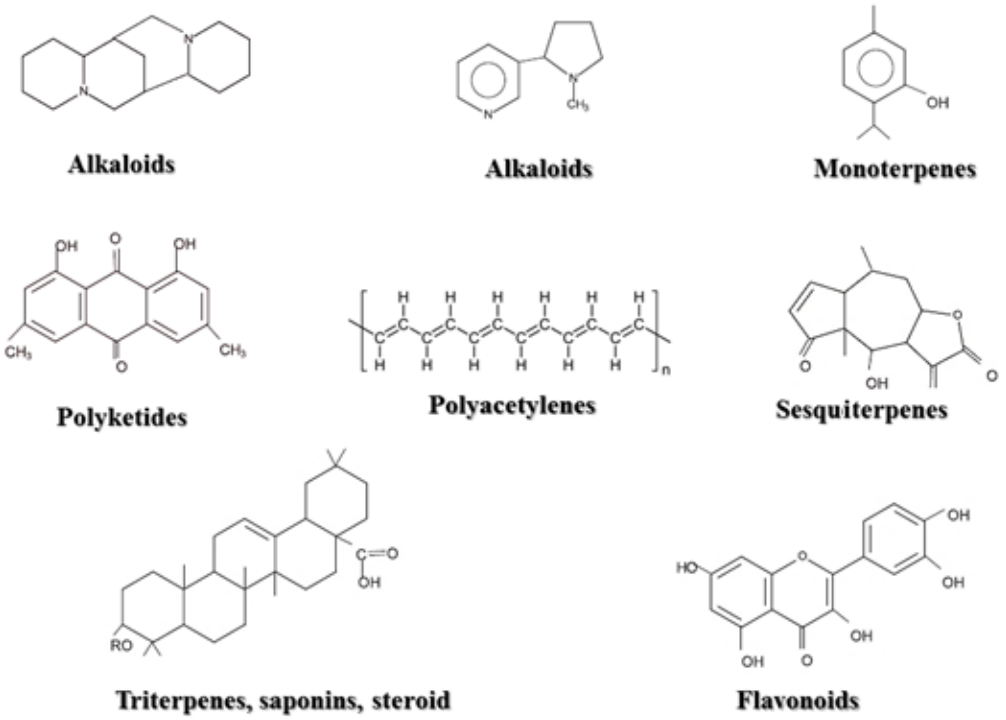
metabolites, which are chemicals that include amino acids, carbohydrates, proteins, and lipids to promote growth and development. Another category is secondary metabolites, a class of chemicals other than primary metabolites that are thought to aid plants in improving their capacity for survival and overcoming local obstacles by enabling them to interact with their environment (Azmir *et al*., 2013). To put it another way, secondary metabolites are metabolites that are regularly created during a growth phase, contain distinctive chemical structures, are typically created as mixes of closely related members of a chemical family, are produced by particular, limited taxonomic groupings of microorganisms, and have no function in growth (although they may have a function in survival) (Erb & Kliebenstein, 2020). Different species primarily choose which secondary metabolites to produce based on their evaluation process and their unique requirements. For instance, flower species provide a scent to entice insects for pollination and fertilization, whereas diseases and herbivores have evolved to produce harmful chemicals to inhibit the growth of nearby plants (Dudareva & Pichersky, 2000). Some of these secondary metabolites are thought to be bioactive because they have an impact on biological systems. The direct definition of plant bioactive compounds is the secondary plant metabolites that have toxic or pharmacological effects on humans and animals (Bernhoft, 2010)

3. SYNTHESIS AND CLASSIFICATION OF BIOACTIVE SUBSTANCES

The classification of bioactive substances into several groups is still arbitrary and depends on the classification's purpose. For instance, pharmacological categorization will not match the scope of categories used in biosynthesis to simplify the description of biosynthetic pathways. According to (Croteau *et al*., 2000), the three major kinds of bioactive chemicals found in plants are (a) terpenoids and terpenes (about 25000 varieties), (b) alkaloids (about 12000 types), and (c) phenolic substances (about 8000 species). Figure 1 provides the general architectur of many kinds of bioactive chemicals.

The bulk of bioactive substances falls into one of several families, each of which has unique structural traits resulting from the manner in which they are constructed in nature. There are four basic mechanisms for the synthesis of bioactive substances, or secondary metabolites: The four processes are presented in order: malonic acid pathway, non-mevalonate (MEP) pathway, shikimic acid pathway, and mevalonic acid pathway (Taiz & Zeiger, 2006). Aromatic amino acids (which derive from the shikimic acid pathway) and aliphatic amino acids both create alkaloids (which are produced by the tricarboxylic acid cycle). Malonic acid and shikimic acid pathways are used to create phenolic chemicals. Terpenes are generated via the MEP and mevalonic acid pathways.

Figure 1. Some types of bioactive chemicals.



4. METHODS USED FOR BIOACTIVE COMPOUND EXTRACTION

It is vital to develop a standardized and comprehensive strategy for screening out these compounds that are beneficial to human health in light of the wide variances between bioactive substances and the variety of plant species (Fabricant & Farnsworth, 2001) described a comprehensive strategy for studying medicinal herbs that began with a name collection of regularly use species and culminated with the industry.

Bioactive chemicals can only be further separated, identified, and characterized before going through the proper extraction procedure. It's essential to utilize multiple extraction methods under different conditions to comprehend the selectivity of extraction from various natural sources. Different methods can be employed to extract bioactive compounds, many of which have remained essentially unchanged for hundreds of years. All of these methods share the following goals: (a) extract specific bioactive substances from complicated plant samples; (b) improve analytical methods' selectivity; (c) raising the concentration of selected compounds will boost the sensitivity of bioassays ; (d) change the bioactive compounds into a form that is better suited for separation, and detection; (e) provide a reliable method that is unaffected by changes in the sample matrix (Smith, 2003).

4.1. Classical and Conventional Methods

The most widely used extraction techniques have relied heavily on liquid-solid extraction for a long time. They typically have simple operations and rely on solvents or heat with various polarities.

4.1.1. *Maceration*

To obtain plant extracts, this procedure involves soaking the plant materials (powered or coarse) in a solvent for a period of two to three days at room temperature while stirring frequently. To prevent solvent evaporation at ambient pressure, an extractor is sealed. In order to liberate the soluble phytoconstituents, the method aims to weaken and break down the plant's cell walls.

After a specific amount of time, the mixture is then squeezed by filtration or decantation (Adegbola *et al*., 2017).

The simplest and most popular method is maceration. The extraction procedure in this stationary method depends on the labor-intensive molecular diffusion concept. Maceration ensures that a new solvent is introduced to the surface of the particles for further extraction and that the concentrated solution that has accumulated on their surface is dispersed (Patel *et al*., 2011).

4.1.2. *Digestion*

In this type of maceration, light heating is used to aid in the extraction process. Menstruum can be used more effectively since the active elements in plant material are not affected by temperature (used as a solvent or combination of solvents for extraction). When the moderately raised temperature is not undesirable, it is employed because it increases the menstrual fluid's ability to dissolve solvents (Zhang *et al*., 2018). Although it can get as high as 50°C, temperatures between 35 and 40°C are the most common. The plant portion that needs to be extracted is put in a container with the liquid that has been preheated to the appropriate temperatures and is kept there while being periodically shaken for a duration that can range from 30 minutes to 24 hours. This method is utilized for herbal material or plant parts that include polyphenolic chemicals or poorly soluble components (Liu *et al*., 2006).

4.1.3. *Infusion*

A simple chemical procedure called infusion is utilized to remove volatile plant material from organic solvents that quickly dissolve or release their active components (Liu *et al*., 2006). Similar to maceration, infusion, and decoction, it entails the process of soaking the plant material in cold or hot water and letting it steep in the liquid. However, the infusion maceration time is shorter. A rotary evaporator can then be used to separate and concentrate the liquid while it is under vacuum.

Infusion is used in the preparation of tea and is recommended for consumption in conditions such as psychophysical asthenia, diarrhea, bronchitis, and asthma, among others. To facilitate urination, lessen irritation, and lower cholesterol accumulation, *Prunus Africana* (pygeum) bark infusion is used orally in tropical Africa (Stéphane *et al*., 2021).

4.1.4. *Lixiviation (Elution)*

The word "lixiviation" (from the Latin lixivium, "lessive") is derived from this concept. Always use a fresh, new solvent that is either cold or hot throughout the extraction process. Water is used as a solvent during the extraction of the components.

4.1.5. *Decoction*

To obtain plant extracts, the present procedure includes boiling the plant material in water. Convection and conduction are two ways that heat is transported, and the kind of substance that can be recovered from plant material depends on the solvent used (Stéphane *et al*., 2021).

The sample is cooked for a predetermined amount of time in a certain amount of water (15 to 60 minutes). After cooling, straining, and filtering, it is provided the desired volume by adding just enough water through the medication. This technique produces more oil-soluble chemicals than maceration and is appropriate for extracting water-soluble compounds from hard plant materials.

4.1.6. *Tincture*

Plant material is extracted using alcohol in this process. Typically, 1:5 ratios of fresh plant material and ethyl alcohol are used. Because they include alcohol, tinctures can be kept at room temperature without going bad (Liu *et al*., 2016).

4.1.7. *Percolation*

It is carried out using dripping the heated solvent through the plant material at a controlled and moderate rate until the extraction is finished before evaporation (for example, 5-7 drops per minute). Usually, the concentrated plant extracts gather near the vessel's bottom. Consecutive filtration can be carried out using filling the percolator with a new solvent and pooling all the extracts to produce a large amount of extract. When producing tinctures and fluid extracts, this process is mostly utilized to extract active ingredients. Its primary drawbacks include the need for huge quantities of solvents, a time-consuming process, and a potential need for experienced personnel (Liu *et al*., 2006).

4.1.8. *Steam distillation and hydrodistillation*

Typically, volatile chemicals, such as essential oil, which are insoluble in water, are extracted from a variety of aromatic and medicinal plants using steam and hydrodistillation techniques. After vapor condensation, the plant products are boiled in water to produce EOs.

Steam distillation takes place at a temperature below the materials' boiling points. The technique works well with thermos-sensitive bioactive substances, such as naturally occurring aromatic compounds. The target compound can then be released from a matrix as a result of the heat causing pore rupture in the sample. According to Raoult's law, the boiling point will decrease when two immiscible liquids are combined. As a result, the mixture's evaporation will approach that of the water in a mixture of volatile chemicals with boiling points range of 150-300°C and water with a boiling temperature of approximately 100°C (Carroll *et al*., 2009; Afroz *et al*., 2015). The fundamentals of steam distillation and hydrodistillation are comparable. In a nutshell, plant material is dissolved in water or a suitable solvent, then heated in the alembic to boiling under air pressure. After liquefying EOs vapors and water in a condenser, the condensate is collected in a decanter, and the EOs are then separated from the water/solvent. Isotropic distillation is the foundation of the extraction theory. The three primary kinds of hydrodistillation are water immersion, direct vapor injection, vapor injection, and hydrodistillation with immersion in water. The amount of time needed for distillation varies on the type of plant material (Afroz *et al*., 2015).

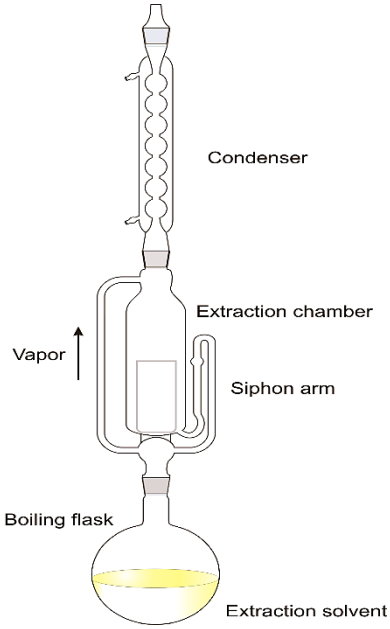
4.1.9. *Soxhlet extraction, continuous hot extraction, or soxhletation*

The Soxhlet apparatus is used in this approach, which involves placing a porous bag or "thimble" containing the finely powdered sample in the thimble chamber. The porous bag is produced from cellulose or sturdy filter paper. Franz von soxhlet created the first soxhlet apparatus in 1879 (Figure 2) (Meyers *et al*., 2004). Warming the extraction solvents in a flask with a flat bottom causes them to evaporate into a sample thimble, which they then condense in a condenser and drip back into. The operation is repeated when the liquid content is drained back into the bottom flask when it reaches the siphon arm (Stéphane *et al*., 2021). The drawbacks include the inability to stir and the need for a significant volume of solvent. Because thermolabile chemicals may be degraded by extended exposure to heat (large extraction times), this approach is not appropriate for them. It is a recognized traditional approach for figuring out how much fat is in various foods (Hemmami, Seghir, *et al*., 2020). The most notable drawbacks of this procedure are exposure to dangerous and combustible liquid organic solvents, and the extremely pure extraction solvents required may increase costs. Additionally, the Soxhlet gadget is unable to provide shaking or stirring to quicken the process (Lee *et al*., 2014).

Comparatively speaking, it uses less solvent than maceration does. Additionally, only one batch of the warm solvent is recycled rather than numerous portions going through the sample (Jha & Sit, 2022; Zeghoud *et al*., 2023). Additional benefits of this method include its straightforward operational mode, its adaptability to higher temperatures that speed up the kinetics process, its low initial investment cost, the lack of filtering, and the solvent's constant

contact with the sample. With heat from the distillation flask, it keeps the extraction temperature comparatively high (Lee *et al*., 2014; Medina-Remón *et al*., 2017; Zhang *et al*., 2018).

Figure 2. Representation of the Soxhlet extraction apparatus.



4.1.10. *Serial exhaustive extraction*

It is a common extraction technique that entails a series of extractions using different solvents that get polarized as they get more polar. The goal is to make it possible to extract chemicals with several ranges of polarities (Stéphane *et al*., 2021).

4.1.11. *Fermentation (aqueous-alcoholic extraction)*

Some pharmaceutical treatments use fermentation as a method of obtaining active ingredients. The crude medication, which can be either a powder or a decoction, is soaked for a predetermined amount of time during the extraction process. After fermentation, alcohol is produced on-site, making it easier to extract the plant material's active ingredients. As a result, alcohol is produced and acts as a preservative. If the fermentation is going to be done in an earthen jar, the water needs to be heated to a boil first. In large-scale manufacturing, porcelain jars, wooden vats, or metal containers are utilized in place of earthen vessels. There isn't a standard for this technique yet (Chhipa & Sisodia, 2019).

For aromatic plants, methods including expression, effleurage (cold fat extraction), and hydrolytic maceration followed by distillation might be used. Some of the most current extraction techniques for aromatic plants include micro distillation, protoplast extraction, solid phase microextraction, and headspace trapping (Stéphane *et al*., 2021).

These strategies are the simplest and most straightforward. Despite the development of more sophisticated extraction techniques, active plant components are still obtained from plants using the potential of traditional solid-liquid extractions. These techniques are criticized because they use a lot of solvents and take a long time to extract, which can kill certain metabolites. The solvents employed in these soaking procedures are essential. There are numerous other cutting-edge extraction techniques have been created (Stéphane *et al*., 2021).

4.2. Innovative (non-conventional) Techniques

The advancement of extraction technology has been advancing steadily in recent years. They are additionally referred to as modern advanced methods.

4.2.1. *Microwave-assisted extraction (MAE)*

Microwaves have wavelengths between 1 cm^{-1} to 1 m^{-1} and operate in the 300 MHz to 300 GHz area of the electromagnetic spectrum of light (Khennouf *et al*., 2003). Two parallel oscillating fields that make up these waves serve as energy and information carriers.

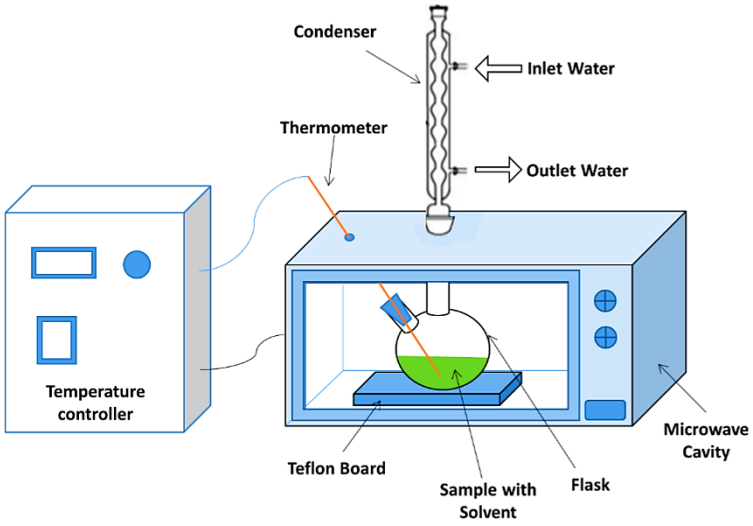
The use of microwave energy speeds up the heating in this extraction procedure. Each molecule is exposed to the microwave field, which has direct impacts such as decreasing temperature gradients, reducing heat-related volume creation, reducing equipment size due to greater process rates, and increasing productivity by making better use of the same equipment's process volume (Gholamhoseinian *et al*., 2012). Given that MAE employs water or alcohol under regulated pressure and elevated temperature, it is a practical green solvent extraction method (Figure 3).

This technique has shown a variety of advantages, including steadiness and ease of handling. According to numerous research, MAE is substantially faster than traditional procedures for extracting active ingredients from plant materials and has greater yields (Adegbola *et al*., 2017; García-Risco *et al*., 2017). MAE can be suggested as a possible replacement for conventional solid-liquid extraction methods. These are a few of the potential benefits: A few milliliters of solvent can be used (need a few milliliters of solvent); The length of extraction might be from seconds to a few min (15 to 20 min); It improves extraction yield; advantageous for thermolabile ingredients; A small plant sample of a few milligrams can be used to extract the trace amounts of heavy metals and pesticide residue; It offers a stirring during extraction, which improves the mass transfer phenomena (Adegbola *et al*., 2017; Nitiéma *et al*., 2018).

Because MAE intensification requires specialized equipment to operate because electricity creates waves, it requires more upfront capital and ongoing operational expenses than traditional approaches (Kumar *et al*., 2010). Banar and colleagues used traditional techniques (maceration, reflux, Soxhlet, hydro distillation, ultrasound- and microwave-assisted extraction (MAE)) with various solvents to extract the bioactive components using Urtica dioica cultivated in Lebanon. According to their findings, MAE was the most successful method. The amount of extracted chemicals was increased while the extraction time was shortened, and less solvent was employed (Sohgaura *et al*., 2018).

When extracting caffeine and polyphenols from green tea leaves for 20 hours at room temperature, MAE achieved a greater extraction yield in 4 minutes (Pan *et al*., 2003). Instead of employing a traditional solvent extraction method for 10 hours, the targeted MAE technique's 15-minute ginsenosides extraction yield from ginseng root was superior (Dhobi *et al*., 2009). Exhibited improved extraction efficiency of MAE when silybinin, a flavonoid from the plant *Silybum marianum*, was extracted instead of more traditional extraction methods, including Soxhlet and maceration.(Jila *et al*., 2011) extracted certain bioactive substances (E-and Z-guggulsterone, tannin, and cinnamaldehyde) under ideal conditions from a variety of plants and demonstrated that MAE is a quicker and simpler procedure than traditional extraction processes. By using MAE, (Chiremba *et al*., 2012). Successfully liberated bound phenolic acids from hard and soft sorghum, as well as maize bran and flour fractions. The solvent concentration, extraction period, and microwave power of the Chinese quince *Chaenomeles sinensis* MAE method were modified to enhance the recovery of phenolics and flavonoids and to improve the extracts' ability to donate electrons (Teng *et al*., 2009).

Figure 3. shows a schematic representation of the microwave-assisted extraction equipment (All rights reserved to (Zghaibi *et al*., 2019)).



4.2.2. *Sonication extraction or ultrasound-assisted extraction (UAE)*

The ultrasound frequencies used in this extraction technique range from 20 to 2000 kHz; this makes cell membranes more permeable and causes cavitation. Despite the process's occasional usefulness, its exorbitant cost prevents it from being used widely. The procedure's primary drawback is the occasionally seen but well-documented harmful impact of ultrasonic radiation on the active ingredients of pharmacological plants, which results in the production of free radicals and, consequently, unfavorable alterations to the drug molecules (Chhipa & Sisodia, 2019). Extraction time, solvent, power, plant material, Liquid/Solid (L/S) ratio, frequency, intensity, and amplitude are all factors that impact how effective UAE is. UAE was superior to other cutting-edge extraction techniques and offered the lowest carbon emissions, greatest mass and heat transmission efficiency, and lowest energy use. According to reports (Iqbal *et al*., 2017), it produced a high level of total phenolic content, antioxidant activity, or particular active chemicals.

Because ultrasonic energy makes it easier for inorganic and organic chemicals to leach by the plant matrix, the main advantage of UAE may be seen in samples of solid plants (Herrera & De Castro, 2005). The most likely process is the enhancement of mass transfer by ultrasound and the rapid access of solvent to plant cell components. The two primary types of physical phenomena involved in the ultrasonic extraction mechanism are (a) Via diffusion mechanism within the cell wall and (b) elution of the contents inside the cell once the walls are broken (Palma & Barroso, 2002). For a successful extraction, the degree of grinding, the sample's level of moisture, solvent, and particle size are all crucial considerations. Additionally, the parameters that control ultrasound action are frequency, pressure, temperature, and period of sonication. Various classical procedures have also been combined with UAE because it is believed that they will heighten the effectiveness of a conventional system. To increase extraction effectiveness, the ultrasonic device in the solvent extraction machine is placed correctly (Hemmami, Ben Seghir, *et al*., 2020).

UAE benefits include a decrease in energy use, extraction time, and solvent usage. Improved blending, quicker energy transfer, decreased thermal gradients, selective extraction, extraction temperature, smaller equipment, quicker reaction to process extraction control, rapid startup, increased output, and the removal of process stages are additional benefits of using ultrasound energy for extraction (Chemat *et al*., 2008).

UAE appears to be a successful extraction method for obtaining bioactive substances from herbal plants (Rostagno *et al*., 2003). Demonstrated the effectiveness of the mix-stirring approach for extracting daidzin, malonyl genistin, and genistin, glycitin, which are four isoflavone derivatives derived from soybean. Depending on the solvent used, researchers discovered that ultrasonic could increase the extraction yield (Herrera & Luque de Castro, 2004). Developed a semi-automatic approach based on ultrasounds to extract phenolic components such as rutin, naringenin, ellagic acid, naringin, quercetin, and strawberry kaempferol using a duty cycle of 0.8 s for the 30s (Li *et al*., 2005) discovered that fresh bark, fresh leaves, and dry bark of Eucommia ulmodies Oliv recovered chlorogenic acid more effectively. By UAE at optimal conditions (20/1 solvent to sample ratio, 70% methanol, and 30 minutes) than conventional extraction procedures (Yang & Zhang, 2008).

4.2.3. *Accelerated solvent extraction or pressurized liquid extraction*

Other names for pressurized liquid extraction include accelerated solvent extraction, pressurized solvent extraction, and improved solvent extraction system (Mukherjee & Patra, 2016). Alternatives to Soxhlet extraction, sonication, maceration, percolation, etc. Dionex Corporation invented pressurized liquid extraction in 1995. It is an automated process that uses a lot of pressures (4 to 12 MPa), high to moderate temperatures (50 to 100 °C) combination with temperatures above their boiling points, and liquid solvents (organic or either aqueous, mixtures, or single) to extract solid materials (Weaver, 2014). When employing water as the extraction solvent, the processes are referred to as hot water extraction, high-temperature water extraction, liquid water extraction, subcritical water extraction, superheated water extraction, and hot water extract pressurized (Mukherjee & Patra, 2016). Solvent, sample size, pressure, pH, temperature, extraction time, and flow rate are all common characteristics that affect the PLE process, with solvent type and temperature being the two most crucial ones *(Barzegar et al*., 2015).

Liquid solvents that are friendly to the environment are employed at moderate to high-pressure levels throughout this extraction process to boost their effectiveness (Sarkar *et al*., 2015). The solubility of the analytes is raised, the matrix-analyte interactions are broken, resulting in a higher diffusion rate, and the extraction process is sped up using raising the solvent's diffusivity. All of these effects are caused by the increased temperature. Contrarily, the increased pressure encourages the solvent to enter the matrix pores while keeping it in a liquid form and preventing it from boiling (Liu *et al*., 2016; Tripathi, 2018).

The key benefits of this method include: (i) a faster extraction time of 15-50 minutes, (ii) the use of fewer solvents (15 - 40 mL), and the absence of filtering. The main drawbacks, however, are expensive equipment and the requirement for continuous variable adjustment to avoid a matrix-dependent efficiency (Sarkar *et al*., 2015). By boosting solubility and mass transfer rates as well as reducing solvent viscosity and surface tension, a higher extraction temperature can help analytes dissolve more readily (Ibañez *et al*., 2012).

PLE was observed to significantly solvent and reduce time consumption use when compared to the conventional soxhlet extraction (Álvarez-Muñoz *et al*., 2004). Today, PLE is being explored as a possible substitute method for supercritical fluid extraction for the extraction of polar chemicals (Kaufmann & Christen, 2002). The extraction of organic contaminants by environmental matrices that are stable at high temperatures is another use for PLE (Wang & Weller, 2006). The extraction of bioactive chemicals by marine sponges has also been done using PLE (Ibañez *et al*., 2012). Research regularly contains applications of the PLE approach for acquiring natural products (Kaufmann & Christen, 2002). Additionally, PLE is widely considered a green extraction process due to the small amount of organic solvent required (Ibañez *et al*., 2012). The use of PLE to extract bioactive substances from various plant sources

has been shown to be successful. Isoflavones were successfully recovered from freeze-dried soybeans using optimal conditions (Shen & Shao, 2005).

Contrasted ASE, Soxhlet extraction, and ultrasonically assisted extraction for the extraction of sterols from tobacco. Due to its quicker process and less solvent use, PLE has been thought of as an alternative to conventional techniques in terms of yield, solvent consumption, and extraction time repeatability. Water solvent at 50-130 °C The ethanol and water mixture (70/30) was weakly effective as a solvent for the extraction of flavonoids from spinach (Howard & Pandjaitan, 2008; Luthria, 2008) revealed that the PLE extraction of phenolic chemicals from parsley (Petroselinum crispum) flakes is affected by solid to solvent ratio factors, static time, pressure, particle size, flush volume, and temperature. Galanthamine and lycorine (Amaryllidaceae alkaloids) were extracted using Narcissus jonquilla using an optimized PLE process, which was more successful than hot-solvent extraction, UAE, and MAE (Mroczek & Mazurek, 2009).

4.2.4. *Supercritical fluid extraction (SFE)*

Supercritical fluids are employed as the extracting solvent in SFE to separate components from the matrix (Ahmad *et al*., 2019).The fluid for extraction that uses CO_2 has various benefits. Additional issues include its lower boiling point (31°C) and critical pressure. Additionally, carbon dioxide is cheap, safe, and abundant in nature. However, while being the favored fluid for SFE, carbon dioxide has a number of polarity restrictions. Solvent polarity is important for extracting polar solutes and when there are significant analyte matrix interactions.

Environmental samples, pesticides, food and perfumes, polymers, essential oils, and natural items all find wide use for SFE (Chhipa & Sisodia, 2019).

The essential oil of rosemary (Rosmarinus officinalis) was extracted using S-CO_2 extraction, steam distillation, and hydro distillation by Conde-Hernández and associates. They discovered that SFC extract had better essential oil yields and antioxidant activity than the other two techniques (Pandey & Madhuri, 2010).

Donn *et al*., (2022) used SFE at 313–343 K temperature and pressure ranging from 14–24 MPa to extract the purine alkaloids (theobromine, caffeine, and theophylline) from Ilex paraguaryensis. At 58.6 °C and 9.5 MPa (Giannuzzo *et al*., 2003), The flavonoid naringin, which is extracted from citrus paradise using ethanol (15 wt.%) with supercritical CO_2 modified, yielded higher yields than pure supercritical CO_2. Grape seeds were used to extract procyanidins and polyphenols using SFE, and more than 79% of the catechin and epicatechin from the seeds were released when methanol-modified CO_2 (40%) (Verma *et al*., 2008) Catharanthus roseus leaves were extracted using SFE-under optimal conditions, and the highest catharanthine recoveries occurred at 25 MPa, 80°C, with 6.6 % methanol acting as a modifier for 40 minutes.

4.2.5. *Pulsed electric field (PEF) extraction*

A method known as pulsed electric field extraction involves exposing vegetable matrix to an electrical potential. An electric pulse produced by a transformer raises voltages from 140 or 220 V to 1000 V or more. This high voltage is transformed in a sealed space with metallic electrodes by a capacitor (Bast *et al*., 2014). The cell degradation and the extraction of components from the intracellular vacuoles are both prevented by this "cold" extraction facilitated by PEF (Jamshidi & Cohen, 2017). Because it may boost mass transfer using breaking membrane structures during the extraction technique, it significantly increases the yield and cuts down on time.

Among the variables that can affect the treatment effectiveness of the PEF extraction are the particular energy input, field strength, and heat treatment.It is referred to as a non-thermal approach that lessens the degradation of the components that are thermolabile (Sy *et al*., 2005).

4.2.6. *Enzyme-assisted extraction (EAE)*

A specialized hydrolyzing enzyme is added throughout the extraction process to perform the EAE, an enzymatic pre-treatment. Micelles are generated in the cell wall structure and cell membrane using macromolecules, including polysaccharides and proteins. The primary obstacles to extracting natural products are high-temperature protein coagulation and denaturation during extraction. Due to the enzymes on the cell wall's hydrolytic activity, membrane, and internal macromolecules, this makes it easier for natural compounds to release, and EAE increases the extraction efficiency. In EAE, hydrolyzing enzymes like cellulose, -amylase, and pectinase are frequently used (Tzanova *et al*., 2020). This method is effective for removing a variety of bioactive materials using plant matrices, although, after filtration, the resulting fraction is abundant in tiny water-soluble compounds, including flavonoids and polyphenols (Tzanova *et al*., 2020).

The release of bound molecules and an improvement in total yield have both been attributed to enzymatic pre-treatment, which has been deemed both unique and efficient (Ghandahari Yazdi *et al*., 2019). By dissolving the cell wall and lipid bodies, the structural polysaccharides are hydrolyzed, adding some enzymes to increase recovery during extraction (Łubek-Nguyen *et al*., 2022). Examples of these enzymes are cellulase, a-amylase, and pectinase. Either enzyme-assisted cold pressing (EACP) or enzyme-assisted aqueous extraction (EAAE) are two methods of enzyme-assisted extraction (Latif & Anwar, 2009). EAAE techniques have typically been developed primarily for the extraction of oils from various t seeds (Sharma *et al*., 2002). Since the EACP system does not have access to polysaccharide-protein colloid, as is the case in EAAE, enzymes are employed to hydrolyze the seed cell wall in this method (Concha *et al*., 2004). It is understood that a number of variables and important factors for extraction include the kind and concentration of the enzymes, plant material particle size, the ratio of solid to water, and the length of the hydrolysis process. According to (Niranjan & Hanmoungjai, 2004), plant materials' moisture content has a significant role in the enzymatic hydrolysis process. Because it is nontoxic and nonflammable (Azmir *et al*., 2013) characterized EACP as a perfect alternative for extracting bioactive components from oilseed, it was discovered that oil extracted using enzyme-assisted techniques included more free fatty acids and phosphate than oil extracted using conventional hexane methods (Azmir *et al*., 2013). Because it substitutes water for organic chemicals as the solvent in the extraction of bioactive substances and oil, the EAE is acknowledged as an environmentally beneficial method (Puri *et al*., 2012) EAE of phenolic antioxidants from grape pomace was examined during the manufacturing of wine by (Meini *et al*., 2019) who discovered a link between the yield of total phenols and the degree of enzyme-mediated breakdown of plant cell walls. (Landbo & Meyer, 2001) found that utilizing different enzymes increased the release of phenolic chemicals from Ribes nigrum pomace. The recovery was maximum with celluzyme MX when (Li *et al*., 2006) EAAE was used to determine the total phenolic content of five citrus peels (Yen Ben lemon, grapefruit orange, mandarin, and Meyer lemon). Another important study conclusion was that higher enzyme concentration significantly improved phenolic antioxidant extraction.

4.2.7. *Turbo-distillation extraction or turbo-extraction* (thrombolysis)

Martel developed turbo-distillation in 1983, and it has been utilized commercially by a number of businesses to extract EOs from tough matrixes (like bark, wood, and seeds). Turbo-extraction, also known as based on extraction, such as turbolysis during agitation and concomitant particle-size reduction. High shearing forces cause cells to rupture, which causes

the active ingredients to dissolve quickly. The plant material is almost entirely depleted and the extraction process takes minutes to finish. Turbo-distillation minimizes extraction time and energy consumption in comparison to hydrodistillation and reduces the degradation of volatile elements (Puri, 2002).

Martins *et al*., (2017) investigated the turbo-extraction of rebaudioside stevioside and stevioside from dried and powdered leaves of *Stevia rebaudiana*. Applying a fractional factorial design to the extraction process allowed researchers to assess the main factors affecting the yield of these glycosides, including the size of the drug powder, the weight ratio of the solvent to the drug, temperature, time, and stirring. Their research showed that turbo-extraction offered hope for the extraction of Stevia rebaudiana glycosides.It sparked a fresh investigation into the extraction of these extracts, which turned into a lucrative industry for emerging nations like Brazil and India (Prakash & Gupta, 2005).

The essential oil obtained using turbodistillation in 30 minutes was demonstrated by Perino and associates to be qualitatively (aromatic profile), and quantitatively (kinetics profile and yield) comparable to that obtained by traditional hydrodistillation in 3 h. They came to the conclusion that this method, which resulted in a shorter extraction time, was ideal for extracting hard matrixes (Puri, 2002). Compared to dynamic maceration, it may be favorable.

4.2.8. *Counter-current extraction* (CCE)

In this procedure, a fine slurry is created from the raw material, which is moist. The target material is delivered just one way within a cylindrical extractor (often as a fine slurry), where it comes into contact with the extracting solvent. Additionally, the initial component shifts, producing a more concentrated extract. In other words, full extraction is feasible when the material quantities and solvent flow rate are adjusted. When high temperatures are used, the procedure is exceedingly effective, quick, and risk-free. Finally, the extracts exit the extractor suitably concentrated at one end, while the residue exits at the opposite end (Chhipa & Sisodia, 2019). His extraction method has many benefits, including A unit amount of the plant material may be extracted using a lot less solvent than with other techniques, including percolation, decoction, and maceration; Since CCE is typically carried out at 25°C, the thermolabile ingredients are not exposed to heat as they would be in most other procedures; Since the medication is crushed in moist circumstances, water dissipates the heat produced during comminution. By doing this, components' thermal damage due to heat exposure is once again prevented; CCE is recognized as more effective and efficient than continuous heat extraction.

4.2.9. *Solid-phase extraction*

In order to chemically separate the various components using the sample preparation method known as solid-phase extraction (SPE) uses solid particles frequently found in cartridge-type devices as chromatographic packing material. Nearly often, samples are in a liquid form (even if certain samples can be used for unique purposes in the gas phase). This technique allows for the separation of dissolved or suspended compounds from other compounds in a liquid mixture based on their physical and chemical properties. Since the chromatographic particles are solid while the sample is liquid, the proper term for this process is "Liquid-Solid Phase Extraction" (Cohen, 2014).

SPE offers several advantages, but four crucial advantages demand special attention: chemical purification and the simplification of complicated sample matrices; minimizing ion enhancement or suppression in MS applications; capacity to fractionate sample matrices for compound class analysis; and traces of extremely low-level chemical concentrations. The solute molecules are preferentially linked to several types of cartridges and disks with varied sorbents in this rapid, economical, and sensitive technique.

4.2.10. *High-voltage-assisted extraction*

With the exception of the electrical discharge occurring through a tiny point, the operation of this equipment operates on a similar concept as PEF. For this, a ground electrode plate is converted into a release from a needle electrode.

In terms of excellent yields, a lot of selectivities, reduced solvent usage, and accelerated extraction times, these so-called greener approaches frequently outperform conventional ones. They are also discovered to be environmentally friendly because they utilize less organic solvents and energy. The literature (Biswas & Biswas, 2005; Vaidya, 2011; Bhateja & Arora, 2012; Lyons *et al*., 2018) describes the combination of extraction techniques to produce high yields overall or high-purity extracts. Its primary benefit is continuous mode operability; this, from an industrial and commercial standpoint, is essential (Bast *et al*., 2014).

4.2.11. *Phytonics process*

In contrast to conventional methods, a novel solvent based on hydrofluorocarbon-134a and a new technique to enhance its exceptional capabilities in the extraction of plant matter provide considerable environmental benefits as well as safety and health benefits. Advanced Phytonics Limited created and patented the "phytonics process" technology (Manchester, UK). The materials typically extracted by this procedure include fragrant elements of EOs and phytopharmacological or biological extracts that may be employed directly without the need for further chemical or physical treatment. The extraction of plant material has been done using the characteristics of the new generation of fluorocarbon solvents. 1,1,2,2-tetrafluoroethane, also known as hydrofluorocarbon-134a (HFC-134a), is the main component of the solvent and has a boiling point of -25°C and an ambient temperature vapor pressure of 5.6 bar. It is non-toxic and flammable. More significantly, this chemical was created to replace chlorofluorocarbons since it does not harm the ozone layer. Using most measures, this is a subpar solvent that cannot dissolve plant waste. The ability to modify the solvents makes the method beneficial since it may be designed to be very selective in extracting a particular class of phytoconstituents by employing modified solvents with HFC-134a. Likewise, different modified solvents may be used to extract a wider range of components. This technique produces biological products with very little residual solvent residuals are consistently less than 20 parts per billion and below detection thresholds. Therefore, the selected solvents have a limited potential for interacting with plant matter and be non-acidic and non-alkaline. The processing facility is sealed at the conclusion of each manufacturing cycle to ensure ongoing recycling and complete recovery of solvents. These devices only operate with electricity, and even then, they use relatively little energy. There is no room for solvents to escape, and even if they did, those that did not include chlorine would not constitute a hazard to the ozone layer. Dry and "eco-friendly" to handle, the biomass that these plants produce as trash.

The phytonics technique is frequently used in biotechnology to extract ingredients for food, beverages, flavored oils, and pharmaceuticals (for example for the synthesis of antibiotics). Producing superior pharmaceutical-grade extracts, pharmacologically active intermediates, phytopharmaceuticals, and antimicrobial extracts are only a few applications for it. Its usage in all of these domains, nevertheless, precludes its application in other areas. From various kinds of plant material, the method is being utilized to extract premium essential oils, aromatic oils, oleoresins, tastes, and natural food colors. The method is also used to refine raw materials that come from other extraction procedures. It offers extraction free of impurities like wax. Numerous biocides are removed from polluted biomass with its assistance (Chhipa & Sisodia, 2019).

4.3. Liquid–Liquid Extraction (Partitioning)

The most frequent next step after the solids have been extracted and the desired organics have been released into the extraction solvent is a liquid-liquid extraction, which takes the positive of combining two (or, sometimes, three or more, which can create two periods) non-miscible solvents, like ether, and water. Polar chemicals should be dissolved in polar solvents, according to the general rule (For instance, proteins, carbohydrates, and amino acids all persist in water). On the other hand, the nonpolar elements frequently stay in the organic phase (Examples include extracting steroids, waxes, terpenoids, and carotenoids into a solvent like ethyl acetate).

When extracting plant material using traditional or cutting-edge techniques, it's critical to reduce interference from substances that might coextract with the target molecules. Additionally, it is necessary to prevent extract contamination, the decomposition of important metabolites, or the formation of artifacts as a result of the extraction process or solvent impurities (Guaadaoui *et al*., 2014); the extracted solution must be filtered to get rid of any particulates, regardless of the extraction technique utilized. Plant extract shouldn't be kept in the solvent for an extended period of time at 25°C or in direct sunlight because of the increased danger of artifact creation and the breakdown or isomerization of extract components that go along with it (Guaadaoui *et al*., 2014).

4.4. Extraction of Specific Metabolites

Before doing additional chromatographic analysis, the chemical research profile of a plant extract and fractionation of a crude extract is useful for separating the principal classes of components from one another. One method based on variable polarity may be used in a plant that produces alkaloids. From plant to plant, the kind and quantity of components that must be divided into various fractions will differ. When labile chemicals are being examined, this approach might be changed (Sy *et al*., 2005).

4.4.1. *Extraction of essential oils* (EOs)

All plant organs generate essential oils; they have a strong perfume and are concentrated aromatic hydrophobic greasy volatile liquids (Gülçin *et al*., 2004). In order to extract them from raw materials, a number of extraction techniques are used, including hydro diffusion, Soxhlet extraction, cold pressing, pressure-assisted expression, or solvent extraction, often known as the scarification technique, gravity, microwave-assisted extraction, microwave hydro diffusion, and water or steam distillation extractions. The easiest extraction method to utilize will rely on how easily the target components will evaporate (volatility) and whether they will be hydrophilic or hydrophobic (polarity) (Liu *et al*., 2002; Agarwal & Rangari, 2003; Howes *et al*., 2003; Triveni *et al*., 2012; Wani *et al*., 2012). However, Soxhlet, hydro distillation, and SFE are the three methods used to extract Eos most commonly applied (Francis *et al*., 2002). The chemical makeup of EOs is considerably influenced by the extraction technique used (Gülçin *et al*., 2004). According to a recent study by Benmoussa *et al*., To improve both the quality and the quantity of the Eos extracted from medicinal and fragrant plants, microwave hydro diffusion and gravity appear to be a quick procedure, a green technology, and a sought alternative technique (Triveni *et al*., 2012).

4.4.2. *Extracting oils and fats*

A large class of non-polar molecules known as lipids are soluble in an organic solvent, for example, diethyl ether, n-hexane, alcohol, or chloroform but are only weakly or totally soluble in water (Kalidas & Mohan, 2010). In contrast to oil, which is a triglyceride that is a liquid or transparent liquid at room temperature, room temperature, triglycerides that make up fats are solid or semi-solid. The degree of solubility, however, determines their chemistry. Vegetable, animal, and marine sources of fats and oils are all possible (Shrinet *et al*., 2021). Pre-treatment

is the first step in a series of procedures needed to produce oilseeds and fats. Before extracting the oil using solvents, it is frequently required to dry, as several organic solvents are not water-miscible and do not readily penetrate the matrix; extraction is ineffective (Hewavitharana *et al*., 2020). Several different types of non-polar molecules, known as lipids, are Because of the makeup of the matrix. Processing techniques are typically neither lipid-specific nor 100% effective at recovering lipid particles. Because they are comparatively non-polar and can thus extract the majority of nonpolar components from crude fat, petroleum ether and diethyl ether are preferred solvents in this situation (Kalidas & Mohan, 2010).

The extraction of edible oils can preserve tocopherols, stop chemical changes in the triacylglycerol, and have adverse effects on taste, appearance, stability, or dietary value. Various traditional and cutting-edge methods, like hot water extraction, microwave-assisted extraction, solvent extraction, cold pressing, supercritical fluid extraction, and high-pressure solvent extraction can be used to extract fats and oils from plants (Shrinet *et al*., 2021). Leaching, washing, diffusion, and dialysis are only a few of the methods used in the extraction of oil (Kalidas & Mohan, 2010). Crude oil is produced from palm oil (seeds of Elaeis oleifera) after being digested and then a pressing stage. In order to release the palm oil from the fruit, digestion aids in the rupture or breakdown of the oil-bearing cells (Gholap & Kar, 2003). Enzyme-assisted extraction is an effective way to increase lipid extraction from a variety of biomasses, including microalgae, soybean, and sunflower (Hegde *et al*., 2014). The three primary adverse effects associated with oil processing are the creation of trans fatty acids, (ii) cis-trans isomerization, and (iii) physical loss (Shrinet *et al*., 2021). To avoid fungus growth and the formation of lipase, which would increase the amount of free fatty acids, oilseeds must not have moisture levels over a particular level prior to processing (Kalidas & Mohan, 2010).

4.4.3. *Volatile organic compounds*

Aromatic chemicals called volatile organic compounds (VOCs) are released by plant tissues. A wide range of VOCs can be produced by plants. They are in charge of giving some dried plants, such as *Camellia sinensis* tea, its distinctive scent. VOCs can consequently be utilized to determine the quality of tea (Shiny *et al*., 2013). As a natural defense against disease and arthropod assaults, many VOCs are released (George *et al*., 2007). In order to extract VOCs, a variety of processes are used, like microwave-assisted hydro-distillation, hydro-distillation, steam distillation, simultaneous distillation solvent extraction, supercritical fluid extraction, solid phase microextraction, purge, and trap (Mallikharjuna *et al*., 2007).

Verde and partners carried out research to define the volatile components and maximize the MAE of the volatile oil terpenes using *Pterodon emarginatus* fruits. According to their study, MAE might be used to extract volatile oils from plants without the use of organic solvents. They demonstrated that even a small amount of water might produce results in extraction. This environmentally friendly technology seems to be a great substitute for extracting terpenes from fragrant plants (Stéphane *et al*., 2021).

All of these methods have advantages and disadvantages that we mention in Table 1.

Table 1. summarizes the pros and cons of the most important extraction methods (Bitwell *et al*., 2023; Rasul, 2018).

Extraction methods	Advantages	Disadvantages
Maceration	- simple process employing straightforward tools and equipment. - No skilled operator is necessary. - process for saving energy. - Ideal simply requires lengthy contact with solvent. - Method that works well for inexpensive, less powerful medications.	- The medication wasn't completely extracted. - It takes a long time and moves quite slowly.
Digestion	- A technique that involves a little warmth during the extraction process and is comparable to maceration. - To prevent the bioactive phytochemicals in the provided plant material from changing due to temperature.	- As a result of heat, the extraction solvent is used more effectively. - To begin the extraction process, the necessary plant parts are added to a container containing the right solvent that has been preheated to the specified temperatures.
Decoction	- Effective for extracting chemicals that are heat stable. - This procedure doesn't call for more complex or expensive tools. - It is simple to carry out.	- The extraction of heat-sensitive compounds is not recommended.
Percolation and Infusion	- Less time-consuming than maceration. - It may be able to extract elements that are thermolabile. - Appropriate technique for expensive and strong medications. - Quicker and more thorough extraction.	- Takes longer than soxhalation. - Additional solvent is needed. - A skilled individual is needed. - Throughout the procedure, particular attention should be paid to the material's particle size.
Steam distillation and hydrodistillation	- Increased oil yield. - The volatile oil's constituents are less prone to hydrolysis and polymerization. - The consistency of the oil quality obtained by steam and water distillation is higher. - This procedure is economical and environmentally benign because no organic solvent is required.	Complete extraction cannot be accomplished. - Some essential oil compounds may hydrolyze as a result of hot water's prolonged action. - It is challenging to manage the temperature, which could cause fluctuating distillation rates. - The method is not profitable.
Soxhlet	- Significant quantities of plant materials can be harvested simultaneously. - Able to use solvent repeatedly - This technique does not call for filtration following extraction. It is a really straightforward procedure. - The repeated interaction of a new solvent with the solid matrix to shift the transfer equilibrium.	- Because the samples are heated to a high temperature for a considerable amount of time, there is a chance that certain compounds will be thermally destroyed. - The extraction procedure requires a lot of labour and takes a long time. - Only a few variables can be manipulated by the process.

5. METHODS OF PLANT BIOACTIVE MOLECULE ISOLATION AND PURIFICATION

Recently, new developments have been made in the separation and purification of plant-based bioactive substances (Brusotti *et al*., 2014). This innovative method enables a comparison between the availability and development availability of various complex bioassays and the availability of precise separation, isolation, and purification methods. Finding a technique that is suitable for screening the source material for bioactivity, such as antibacterial, antioxidant,

or cytotoxicity, while combining simplicity, specificity, and speed, is the aim when looking for bioactive substances (Altemimi *et al*., 2017).

Because animal tests are more costly, time-consuming, and subject to ethical debates, in vitro procedures are typically preferred over in vivo trials. Finding definitive methodologies or protocols to extract and identify certain bioactive materials is difficult due to a number of variables.

This could be because a plant has several components (tissues), many of which create very different compounds, and because the bioactive phytochemicals have a variety of chemical structures and physicochemical characteristics (Kumar *et al*., 2021). The selection and collection of plant materials are regarded as the first steps in the process of isolating and identifying a bioactive phytochemical. The last stage is retrieving ethnobotanical data to identify potential bioactive compounds. The active substances that are responsible for the bioactivity can subsequently be isolated and purified by creating extracts using a variety of solvents.

Techniques for column chromatography can be utilized to separate and purify the bioactive components. The purification of the bioactive molecule is sped up by modern technologies like High-Pressure Liquid Chromatography. The purified chemicals may be recognized using a variety of spectroscopic methods, including UV-visible, mass spectroscopy, Nuclear Magnetic Resonance (NMR), and Infrared (IR) (Manthey & Guthrie, 2002).

5.1. Purification of the Bioactive Molecule

The use of column chromatography and paper thin-layer techniques has allowed for the isolation and purification of several bioactive materials due to their availability in a variety of fixed phases, affordability, and ease, column chromatography and thin-layer chromatography (TLC) are still frequently used(Bajpai *et al*., 2016).

For separating the phytochemicals, alumina, silica, polyamide, and cellulose are the most valuable materials. High concentrations of complex phytochemicals found in plant materials make good separation challenging (Altemimi *et al*., 2017). Therefore, for very valuable separationsit is advantageous to increase polarity utilizing many mobile phases. The fractions of compounds using column chromatography have traditionally been analyzed using thin-layer chromatography. Some analytical methods have been utilized for the separation of bioactive material, such as TLC and silica gel column chromatography (Annadurai, 2021).

6. CONCLUSIONS AND FUTURE PERSPECTIVES

The constant need to extract plant bioactive components stimulates research for convenient extraction methods. The creation of the majority of non-conventional extraction procedures is largely due to the growth of chromatography advancement and awareness about the environment. However, since most of these approaches are based on diverse mechanisms and extraction improvement is the consequence of several processes, knowing every component of the non-conventional extraction process is essential, and It is important to look into the incorporation and development of hybrid approaches while taking into account the properties of the plant material and compounds selection. Some of the current approaches still don't have enough experimental data. The assessment of extraction efficiency is influenced by the proper use of standard methods. However, the rising economic significance of bioactive compounds and the commodities that include these substances may encourage the development of more advanced extraction techniques in the future.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Imane Ghenabzia: Conceptualization, Data curation, Writing-Original draft preparation, Writing- Reviewing and Editing. **Hadia Hemmami**: Writing Reviewing and Editing, Visualization, Supervision. **Ilham Ben Amor**: Data curation, Writing-Original draft preparation, Writing- Reviewing and Editing. **Soumeia Zeghoud**: Data curation, Writing-Original draft preparation, Writing, Reviewing and Editing. **Bachir Ben Seghir**: Data curation, Writing-Original draft preparation, Writing- Reviewing and Editing. **Rokaia Hammoudi**: Data curation

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